Biology and Biotechnology

Our research in biology and biotechnology serves to improve human health, advance fundamental bioscience, and minimize health care costs through improved prevention and diagnosis of illness and more effective delivery of health care.

A formal biomedical and environmental research program was first established at LLNL in 1963 to study the radiation dose to humans from radioactive releases into the environment. Primary concerns were from underground weapons testing and Plowshare applications. Since then, the program has responded to changing national needs and new technologies. We still study the environment's effects on humans, but our interests have broadened to include other important national health issues.

Today, the Biology and Biotechnology Research Program provides a scientific foundation for understanding the health consequences of the environment and our use of various energy resources. As our name implies, we develop technological solutions to problems related to health assessment and fundamental biology, usually at the genetic or molecular level. Our research is based on the long-term goals of the DOE, particularly the Office of Health and Environmental Research.Within the Laboratory, which is undergoing a dramatic transition in emphasis because of changing international conditions, biology and biotechnology research is identified as a major growth area.

Scientist Bart Beeman digitizes an ethidium-bromide-stained agarose gel with a two-dimensional gel image reader, an alternative to capturing the images on Polaroid film. These gels are used for routine DNA analysis.

Our Mission

The missions of the Biology and Biotechnology Research Program are to initiate and conduct top-quality basic and applied research in the health and life sciences in support of national objectives; develop and sustain a multidisciplinary, talented, and motivated research team; and ensure the transfer of our knowledge, science, and technology to all sectors, including industry, universities, and the general public.

We strive to serve our customers well in performing our mission. The agencies who fund our work include both industry and our traditional sponsors—the DOE and other federal agencies. We regard these customers as true partners in our research, and we seek feedback from them, our scientific peers, and the public on our direction and performance. We also strive to play a special role in educating the public regarding the scientific basis of our research and the ways in which it benefits them.

Our Vision of the Next Century

Our planning is driven by our vision of biology and biotechnology as the revolution of the 21st century. A primary driver is the international Human Genome Project, its extensions to other species, and its applications to improving health, agriculture, the environment, and the economy. The DOE has played a founding role in this initiative, and LLNL has been involved from the outset. We aim to retain our existing expertise in cellular and molecular biology of DNA, as well as strengthen other areas for future needs. With that framework, we will enter the next century equipped with a creative, flexible, and diverse workforce able to confront changing scientific priorities.

Building for the next century, we will add at least three additional competencies: assessing protein structure and function, characterizing human variation in relation to induced mutation and disease, and applying our existing expertise in the health sciences, physical sciences, and engineering to develop new, cost-effective, health-care technologies. The first two competencies will draw on the information, materials, and technologies developed by the human genome project. The third

will depend upon the expertise of the entire Laboratory, especially skills that were first developed in the defense programs. We expect these competencies to enable us to extend the scope of our research to agriculture and the environment.

Core Competencies and Research Objectives

Our existing core competencies are in molecular genetics and genomics, DNA repair, human mutation assessment, molecular toxicology, bioinstrumentation, and bioinformatics. They are embedded in all our research projects. Our global scientific objectives are to:

- Understand the nature of genetic organization and unravel the genetic code in appropriate organisms as necessary to study the consequences of adverse environments on living systems.
- Identify, isolate, and characterize the genes that can repair DNA damage and understand their role in damage prevention or amelioration.
- Develop and apply methods to assess the risk to humans from exposure to radiation and chemicals.
- Develop and apply biophysical techniques to understand protein structure and function.
- Couple our bioscience strengths to health care needs.
- Develop industrial partners to transfer the results of our science and technology to the commercial sector.
- Transfer our knowledge and experience to industry, the academic world, and the general public through research activities and education at all levels.

Technology transfer has a high priority. We foster a team research environment internally and encourage collaborations worldwide. In the Human Genome Center alone, we maintain well over 150 active collaborations. We also have several interactions with industry, many of which have resulted in licenses, material transfers, or CRADAs. Collaborative research areas include flow cytometry, diagnostic antibodies, DNA

Highlights for 1994

- Continued constructing a high-resolution physical map of human chromosome 19. To date, 80% of the chromosome has been spanned by ordered elements. More than 300 genes and markers, including more than 40 of the highly informative genetic markers, have been assigned to cosmids and localized on the map.

- Cloned a gene involved in the first autosomal linkage. This knowledge is useful in cell surface recognition.

- Developed a method to automatically integrate a dozen types of DNA physical mapping data into a global ordered map.

- Completed the sequencing of more than 240 kilobase pairs of human chromosome 19 from cosmids. These include the chromosome regions surrounding three DNA repair genes and the corresponding regions in the mouse genome.

- Completed a high-resolution map for a 700-kilobase-pair region on chromosome 19 encompassing the pregnancy-specific glycoprotein gene family.

- Identified defects (a necessary first step in repairing DNA damage) in one of the XP gene cells from xeroderma pigmentosum group D and sun-sensitive trichothiodystrophy patients.

- Developed a new diode-laser-based flow cytometer, which incorporates a novel detection system and allows for perpendicular and forward light-scatter measurements, potentially useful in a clinical environment.

- Developed a prototype DNA sequencing instrument to increase throughput 40- to 100-fold and signed a CRADA for commercialization.

- Showed that by coupling high-performance liquid chromatography with AMS, we can quantitatively measure metabolites of potential mutagens at levels as low as 10^{-19} moles.

- Developed chromosome painting probes for the mouse and rat for quick and simple microscope analyses.

- Identified the probable cause for the inactivity of the enzyme lactate dehydrogenase.

- Predicted, with computational biological techniques and biophysical measurements, the three-dimensional structure of the PhIP molecule and a segment of DNA containing the adduct.

Molecular biologist Stephanie Stillwagon loads a DNA sample into an automated DNA sequencer as part of the Human Genome Project. Researchers are also working with an industrial partner to build the next generation of such instruments.

primers, chromosome painting probes, electrophoresis, and bioanalysis software.

We have provided materials, know-how, and the use of our facilities to qualified scientists, students, and teachers. In 1994, we sponsored more than 50 students and 35 visiting scientists. Most students enter through our summer program; others are in residence to attain an advanced degree at one of our collaborating universities. Our Institute of Genetics and Genomics is the conduit to the university environment.

We believe that a diverse workforce is a most important investment for the future. In 1993, we received an award from the Director of the Laboratory for our diversity efforts.

Genomics

Genomics is the study of the organization and function of genetic material, that is, DNA. Our interest revolves around the goals of the international endeavor to construct physical maps and sequences for the human genome. We are focusing our attention on developing resources in molecular biology, computations, and instrumentation to support these goals. Our unique contributions include chromosome-specific libraries, fluorescence in-situ hybridization techniques to paint whole chromosomes and measure distances along DNA, mapping software, new DNA-sequencing instrumentation, robotics for laboratory automation, and flow systems for analyzing and purifying cells and chromosomes.

So far, we have concentrated on the construction of a high-resolution cosmid map of chromosome 19. Cosmids are relatively small, cloned fragments of DNA. With this phase of the project nearing completion, greater emphasis will be placed on validating the map, completing the fine-structure mapping, finding the estimated 2000 genes on this chromosome, and determining the sequence of the nucleotide bases, or chemical units, within the cosmids along the chromosome. When we began mapping in 1987, only 55 genes or other types of DNA segments had been linked to chromosome 19. We have now located more than 300 genes or genetic markers on chromosome 19, including the structural defect associated with the muscle disease myotonic dystrophy. As the next step in our long-term effort, we are planning to dramatically increase our efforts in DNA sequencing. We have already sequenced more than 240 kilobase pairs of chromosome 19, including the DNAs and genomic regions associated with three human DNA repair genes.

We consider two major applications of the Human Genome Project to be of particular interest to DOE: structural biology and the characterization of human genetic variation. Consequently, we are working in structural biology to take advantage of the information available from the genome project and other efforts in our program. Identifying all the genes and their protein products should provide an economic incentive for industries interested in developing specific pharmaceuticals. Regarding genetic variation, we have identified unique repeating sequences of DNA that can discriminate among some human groups and can thus be applied in forensic analysis. We are exploring new methods to rapidly assess mutations in humans using automated approaches to determine the underlying DNA sequence. As our technologies advance, we envision applying our mapping and sequencing strengths to microbes of importance in basic research, plants that are valuable in the energy cycle or potential agricultural crops, and animals whose genomes remain unexplored.

DNA Repair

We are especially interested in those genes that increase susceptibility to disease. One family of

such genes includes those that repair DNA damaged by exposure to radiation or chemicals. Repair plays a critical role in reversing environmental damage that can lead to cancer or birth defects, and it is closely intertwined with other aspects of DNA metabolism. DNA can be damaged by strand breaks, oxidized bases, and chemical adducts arising from exposure to sources of ultraviolet and ionizing radiation (including radon) and a multitude of chemicals associated with fossil energy and other technologies. DNA repair acts on these kinds of damage, plays a critical role in the response of tumor cells to radiation and chemical agents, and probably helps to retard the aging process.

As a participant in the international genome study, our goal is to identify and clone the human genes that determine repair. The cloned genes become the key for overproducing and purifying repair proteins so that their biochemical mechanism of action can be determined. We want to understand whether certain repair genes are the rate-limiting factor in cellular resistance to radiation and chemical exposure, although we recognize that there is likely to be wide variability in the repair capacity among individuals. Our major emphases are on identifying new repair genes, purifying and analyzing repair proteins, understanding the role of these proteins in human diseases, and creating new strains of mice (transgenic mice) in order to study human repair disorders.

We are relating the DNA repair genes to human genetic diseases in which repair is faulty, particularly the xeroderma pigmentosum (XP) gene. Xeroderma patients are extremely sensitive to sunlight and are at high risk for skin cancers. We have identified mutations in one of the XP genes (D) of several affected individuals, and we have cloned another gene (ERCC4) that is a good candidate for a different clinical form of xeroderma pigmentosum. We have also cloned a third DNA repair gene, XRCC1. Defects in this gene increase a cell's susceptibility to damage from ionizing radiation.

Molecular Toxicology and Human Risk Assessment

We also are developing techniques to estimate the biologically relevant exposure to genotoxic agents, carcinogens, and mutagens as a step toward estimating risk. The biomarkers or surrogates currently being used in human studies include the classical cytogenetic endpoints of structural aberrations and sister chromatid exchange. The newer chromosome-painting techniques are also being used to identify persistent translocations long after exposure. Using the painting technology for high-speed measurement will allow us to obtain data on more individuals and to estimate the damage following low-dose exposures. Chromosome painting has also been developed for use in mice, further enhanced by multicolor painting techniques. The mouse models should allow us to look at specific factors that affect cytogenetic damage (i.e., metabolism, repair, exposure) that might be manifest in humans as genetic differences in susceptibility.

Mechanical engineer Don Masquelier works on a miniaturized flow cytometer, which uses fiber-optic data collection inside the flow stream. Compared with conventional flow cytometers, this instrument's novel signal-detection system yields a signal that is less noisy and an order of magnitude stronger.

Biomarkers developed at LLNL have been combined with those developed by others in coordinated multiple-end-point epidemiological studies (funded by the National Institutes of Health) to quantify the exposure in several populations, including smokers, pesticide workers, and "liquidators" (cleanup workers) from the Chernobyl nuclear power plant accident. Such studies are becoming more closely linked to health consequences, cancer, and pregnancy outcome. These analytical techniques will continue to be key components of efforts to estimate the potential health consequences of population exposures.

We have enhanced our ability to predict low-dose effects from chemical exposure using LLNL's accelerator mass spectrometer (AMS) and have initiated experiments to couple the measured exposure to biological outcome. This method is sensitive enough for us to measure ^{14}C-labeled adducts following dietary intake of food-mutagens by mice at levels that are equivalent to the normal dietary intake for humans. We have expanded our AMS studies to quantify low-level exposures to

Biomedical scientist Ed Salazar loads DNA samples into an agarose gel for analysis. He is working as part of the DNA Repair Group on a repair gene called ERCC2, which is involved in one form of the disease xeroderma pigmentosum.

benzene. A significant part of these studies is the linking of new technologies such as AMS to traditional separation methods such as electrophoresis. We have used this approach to show that benzene is associated with the spindle fibers needed for DNA replication and may be responsible for cytogenetic damage through its ability to bind specific critical proteins. This finding is possible only because of the ultrasensitivity that AMS brings to these types of measurements. Also, as previously mentioned, many researchers are interested in using AMS technology to monitor low-level presence of chemicals in environmental samples and in humans. This use has been demonstrated for benzene, drugs, heterocyclic amines, and pesticides in rodents and for heterocyclic amines in monkeys.

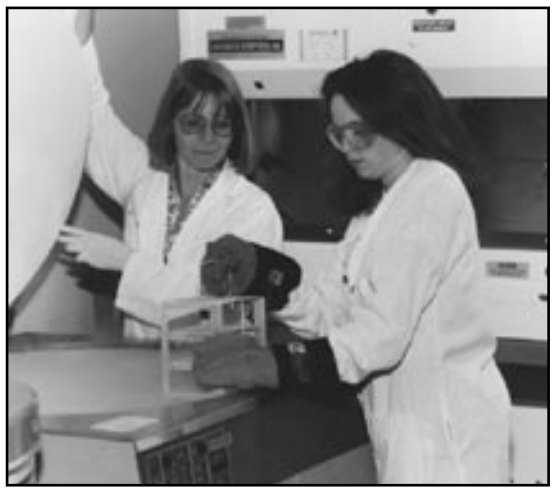
Structural Biology

A new effort in structural biology has been initiated to expand our program's capabilities in three-dimensional structure analysis of proteins, nucleic acids, and the complexes they form with each other and with other molecules. This effort is relevant to DOE's growing interest in structural biology and to the increasing national interest in identifying how defects in molecular structure cause cancer and genetic disease. The research is expected to support the human genome effort by producing the tools needed to predict the structure (and possibly the function) of proteins that belong to many of the gene families for which DNA sequence information is becoming available.

Our initial efforts have focused on resolving the structures of several different types of molecules, including chemical mutagens that bind to and damage DNA and proteins that repair damaged DNA, inactivate entire genomes, and replicate the DNA molecule. Studies of small chemical mutagens such as PhIP, a heterocyclic amine produced when foods are cooked, employ computational and other methods to predict the physical structure of the molecule and reactive intermediates, identify the structure of the complex it forms with DNA, and determine how such molecules damage DNA and cause cancer.

We are using various forms of spectroscopy, computer modeling, and x-ray diffraction to resolve the unusual structure adopted by a small, highly charged protein (protamine) when it binds to DNA, so we can determine how it inactivates all the genes and understand how defects in the process cause male infertility and early fetal deaths. Homologous modeling methods are being used to predict the structure of unusually thermostable enzymes, such as a DNA polymerase isolated from *Thermus aquaticus*, and inactive enzymes, such as the Guaymi Indians' variant of lactate dehydrogenase, and to discover how changes in protein structure modulate its function. In the future, many of these same techniques will be applied to the analysis of a whole series of proteins that repair damaged DNA.

The results of this work will help explain the mechanisms responsible for DNA adduction by small molecules, increase our understanding of how molecular lesions lead to mutations and



Biomedical scientist Cindy Thomas (left) and summer student Sarah Henson remove samples from a liquid nitrogen tank. Viable cells are routinely frozen for later use as part of epidemiological studies to quantify damaging exposures in several populations, including smokers, pesticide workers, and "liquidators" (cleanup workers) from the Chernobyl nuclear power plant accident.

cancer, provide new tools for predicting the structure (and possibly the function) of many proteins of interest to the medical community, advance our knowledge of what structural features of the DNA-protamine complex are essential for male fertility, and provide new information about the molecular structure of the enzymes that synthesize DNA.

Technology Development

During the last year, we have increased our emphasis on technology development, which we see as a mechanism for accelerating all our research. Our technologies focus on aspects of molecular biology (e.g., vector development), molecular genetic applications (e.g., chromosome painting and high-resolution fluorescence in-situ hybridization), software development for database interaction, networking in a client–server mode, and bioinstrumentation. Our emphasis in bioinstrumentation has been to improve methods for analyzing cells and chromosomes by flow systems, image analysis for cytogenetic automation, robotics for automating microbiology and chemistry techniques, and microfabrication technology to create miniaturized chambers for high-throughput chemical analysis and increased throughput and sensitivity of electrophoresis for DNA sequencing. Many of these technologies have been transferred to other laboratories and to industry.

Center for Health Care Technology

Industry and medical facilities are becoming increasingly interested in receiving assistance from LLNL in developing new technologies that can be used in medicine and health care. At the same time, the downsizing of defense programs has created a growing interest on the part of the involved scientists and engineers in applying the expertise developed in defense projects to the health care field. LLNL's Health Care Center, which serves the entire Laboratory, grew out of this mutual interest. Currently funded projects include an NIH-sponsored effort to use x-ray tomography to examine osteoporosis. We also are developing microsurgical tools for insertion in catheters, imaging methods and hardware to improve laparoscopic surgery, innovative hardware and software for mammography, and software and networking tools for medical information management and transfer. Lacking, however, is the coordination needed to minimize redundancy, provide a strong and unified image to potential partners and sponsors, and market ideas and attain long-term funding. The Center was formed to fill this need.

Summary

Our goals have broadened considerably to align with the changing priorities of the DOE and changing national and global needs. Our present programs cover many aspects of biotechnology research, including molecular genetics and genomics, molecular toxicology, instrumentation development, and health care technology development.

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Biophysicist Nick Hud uses sophisticated molecular modeling algorithms to better understand interactions between DNA and other molecules. The results are displayed graphically. Computer modeling is one of several tools used by scientists in the Structural Biology Group.